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TITLE: Novel Biomarker Discovery for Diagnostic and Therapeutic Strategies in Prostate Cancer

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14. ABSTRACT

PURPOSE: to identify high affinity aptamers that distinguish between prostate cancers that are likely to remain organ-confined and those with potential to metastasize. **SCOPE**: This was a pilot project to generate RNA aptamers that selectively react with a prostate cancer cell line that remains confined to the prostate (LNCaP) vs. a subpopulation of this cell line that has acquired the ability to metastasize aggressively, employing Cell-Selex and Aptamer-Facilitated Biomarker Discovery (AptaBiD) technology. **TASKS AND PROGRESS**: (1) Non-metastatic LNCaP-Pro-5 cells, metastasis-prone LNCaP-LN3 cells, and parental LNCaP cells obtained, phenotypically validated and used to screen an RNA 40 bp aptamer library. (2) After 8-12 rounds of Cell-Selex, aptamer pools were screened by flow cytometry against parental, aggressive and non-aggressive LNCaP clones to confirm signal enrichment. (3) Next-generation sequencing and bioinformatic analysis was conducted and candidate RNA aptamers were identified *in silico*. 4 candidates were selected for bulk synthesis and screening. **RESULTS:** 2 related aptamers were found to specifically stain plasma membranes of metastatic LNCaP-LN3 prostate cancer cells in culture. Membrane binding was accompanied by cell death over a period of 3 hours. No binding or cell death was seen with the parental or non-aggressive LNCaP-Pro-5 lines. In frozen sections of xenograft tumors established in immunodeficient NSG mice using each of the prostate cancer lines, the aptamer exhibited strict selectivity for staining of LnCaP-LN3. **CONCLUSIONS AND SIGNIFICANCE:** a novel aptamer identified through Cell-Selex is able to discriminate between metastasis-prone and non-aggressive LNCaP prostate cancer cell lines by binding to an unknown membrane component present only in LnCaP-LN3, both in culture and in xenograft tissue. Binding by the aptamer is cytotoxic. Identification of the aptamer target will help to elucidate the biology underlying metastatic potential in prostate cancer. In addition, this highly specific aptamer offers a tool for a clinical diagnostic study of novel biomarkers of prostate cancer aggressiveness.

15. SUBJECT TERMS
Prostate cancer, Cell-selex, metastasis, prognosis, biomarker, biopsy, screening, prediction, tissue microarray

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. INTRODUCTION:

Prostate cancer (PC) is the most common non-skin cancer diagnosed in American men, with about 1 out of every 7 men receiving this diagnosis during his lifetime. For many men, prostate cancer is an incidental diagnosis that will never require treatment or affect length or quality of life, while for others, PC is a catastrophic, aggressive disease that can metastasize to other tissues of the body well before it becomes symptomatic. Thus, early and accurate detection of PC is essential to the treatment and cure of this disease, and to avoiding both under- and over-treatment. The purpose and scope of this research was to exploit novel aptamer technologies to identify novel tumor-specific markers, which could improve detection of PC, predict tumor aggressiveness and serve as targets for imaging and treatment.

2. KEYWORDS:

RNA, aptamer, cytotoxicity, xenograft, metastasis, bioinformatics, RNA-Seq, next-generation sequencing, plasma membrane, NOD-SCID-Gamma mice, Cell-Selex, flow cytometry

3. ACCOMPLISHMENTS:

The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?

Task 1: Propagate non-metastatic (LNCaP-Pro-5) and metastatic (LNCaP-LN3) prostate cancer cell lines ontrol cell lines to initiate the aptamer screening process. Completed March 2014.
Task 2: Prepare dual-labeled, randomized DNA aptamer pool for AptaBiD selection process. Completed January 2012. Replaced by RNA aptamer library March 2014.
Task 3: Perform first round of "positive" DNA aptamer selection using the non-metastatic (LNCaP-Pro-5) and metastatic (LNCaP-LN3) prostate cancer cell lines, respectively. Completed April 2014.
Task 4: Perform first round of "negative" DNA aptamer selection using parental LNCaP cells, HPECs and non-prostate control cell lines with the enriched DNA aptamers pools. Completed May 2014.
Task 5: The positive and negative selection of DNA aptamers that bind either organ-confined or metastatic LNCaP cells will be repeated for a minimum of 10 cycles. Completed October 2014.
Task 6a. Perform next-generation sequencing on RNA aptamer pools after cycle 8 (replicate samples x 2), using Illumina HiSeq 2000 sequence detection system. Completed December 2014.
Task 6b. Perform bioinformatic analysis on sequence information to identify core sequence motifs of selected RNA aptamers. Completed April 2015.
Task 6c: identify lead RNA aptamer candidates through sequential amplification properties and synthesize lead candidates to scale for testing. Completed December 2015.
Task 7: Expression of new indolent and progressive prostate cancer biomarkers in vivo using fluorescent microscopy and immunohistochemistry Completed March 2016.

See attached report on next pages.

1. Introduction:

Prostate cancer (PC) is the most common non-skin cancer diagnosed in American men, with about 1 out of every 7 men receiving this diagnosis during his lifetime. The American Cancer Society estimates that in 2014, about 233,000 new cases of prostate cancer will be diagnosed, and about 29,480 men will die of prostate cancer. For many men, prostate cancer is an incidental diagnosis that will never require treatment or affect length or quality of life, while unfortunately, for other men, prostate cancer is a catastrophic, aggressive disease that can metastasize to other tissues of the body well before it becomes symptomatic. Thus, early and accurate detection of PC is essential to the treatment and cure of this disease, and to avoiding both under- and over-treatment. There is a critical need to search for novel tumor-specific markers that may enable more accurate detection of PC, predict tumor aggressiveness and serve as molecular targets for imaging and effective therapies. Our research objective is to utilize novel technologies to identify, isolate and characterize high affinity nucleic acid oligomers (aptamers) that distinguish between prostate cancers that are likely to remain organ-confined and those with potential to metastasize. We employed Cell-Selex [1-4] to generate RNA aptamers that are selected to discriminate between PC cell lines that are either indolent or aggressive in their growth properties.

2. Keywords: Prostate cancer, RNA aptamer, polymerase chain reaction, next-generation sequencing, biomarker, metastasis, risk stratification

A. Rationale:

It is critical to be able to identify the subset of prostate cancers that are likely to exhibit aggressive, metastasis-prone behavior. The highly metastasis-prone LNCaP-LN-3 prostate cancer cell was subcloned from a parental cell line (LNCaP) that almost never metastasizes[6, 7]. Thus these constitute a pair of cell lines that are genetically similar (derived from the same individual) but differ in metastatic potential. By identifying differences between these cell lines, we will be able to identify markers of aggressive behavior in prostate cancer.

B. Summary of Achievements on Scope of Work

Task 1: Propagate non-metastatic (LNCaP-Pro-5) and metastatic (LNCaP-LN3) prostate cancer cell lines as well as control cell lines to initiate the DNA aptamer screening process.
Status: Completed.
Task 1a. We have obtained LNCaP-Pro-5, LNCaP-LN3 and parental LNCaP cell lines from Dr. Pettaway of MD Anderson Cancer Center, Houston, TX[6] and American Type Culture Collection (http://www.atcc.org) respectively.
Task 1b. We have propagated and tested these cells for growth properties *in vitro* to confirm that they represent distinctive indolent (LNCaP and LNCaP-Pro-5) and aggressive (LNCaP-LN3) phenotypes. We have used several measures of aggressiveness: tumor doubling time, growth and invasiveness in 1- (scratch assay) and 2- dimensional (Boyden chamber) assays. These assays have indicated that there is little difference between the two indolent cell lines, but substantial difference between these two and the LNCaP-LN3 line, which is able to aggressively metastasize from the prostate *in vivo* (Pettaway ref). We also have propagated multiple controls: MCF-7, MCF-10A, MCF-12A, MDA-MB-231, HUVEC and HMEC cell lines, and primary rat and human fibroblasts, which will be utilized in the negative selection steps. By subtracting against these control cell types, we will eliminate aptamers that cross-react with non-prostate cancer cell types.
Status: Completed.

Task 2: Prepare fluorinated RNA aptamer library for screening indolent and aggressive LNCaP cells. The pool of RNA aptamers is amplified from the general template: TCT CGG ATC

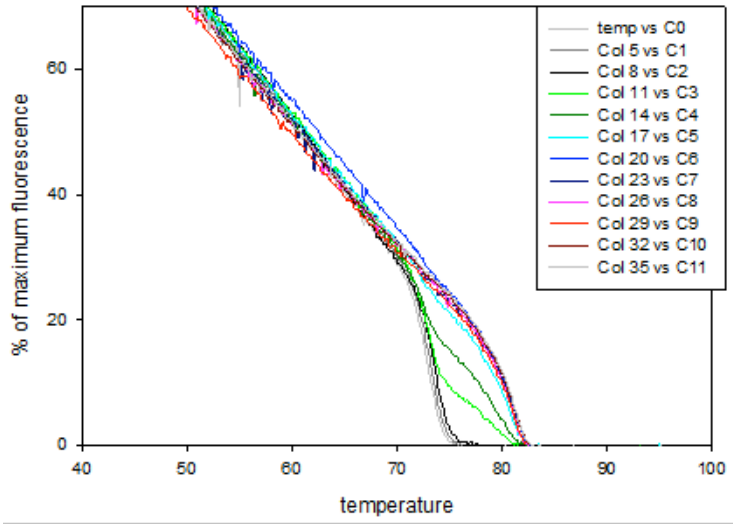

Figure 1. Melt curves for cycles 0-11. With progressive selection and loss of complexity, the RNA pool melting temperature increases.

CTC AGC GAG TCG TCT G-40(N)-CCG CAT CGT CCT CCC TA, (where N represents 40 random nucleotides) and in vitro transcribed Fluorination of the nucleic acid backbone stabilizes the RNA aptamers against RNAse degradation.
Status: completed.

Task 3: Perform first round of "positive" RNA aptamer selection using the metastatic (LNCaP-LN3) prostate cancer cell line.
Task 3a: Initial positive selection. LNCaP-LN3 (aggressive) cells were used as the "positive" selection. Metastasis-prone LNCaP-LN3 cells were incubated with the complete RNA aptamer pool (30 minutes at 4°C) to initially select for RNA aptamers that interact with more aggressive prostate cancer cells.. Unbound RNA aptamers were removed by media aspiration and washing in PBS.
Task 3b: Recovery of positively selected aptamers. Cells were heated (95°C for 5 minutes) to remove bound RNA aptamers.
Task 3c: Amplification of recovered aptamers. Released organ-confined or metastatic prostate cancer cell specific RNA aptamers were amplified via polymerase chain reaction (PCR) to expand this positively selected population.
Status: Completed.

Task 4: **Negative selection.** We performed the first round of "negative" RNA aptamer selection using parental LNCaP cells with the enriched RNA aptamers pools.
Task 4a: Enriched metastatic prostate cell-specific RNA aptamer pools (generated in Task 3) were incubated with cellular pools containing parental LNCaP cells (30 minutes at 4°C).
Task 4b: Bound aptamers were removed by centrifugation of the control cells to which they are bound. RNA aptamers that do not bind to these cells were recovered in the cell supernatant and retained. The retained, "subtracted" RNA aptamer pool are enriched for species that bind to metastatic LNCaP-LN-3 cells but not control cells.
Task 4c: The subtracted aptamer pool is amplified by PCR.
Status: Completed.

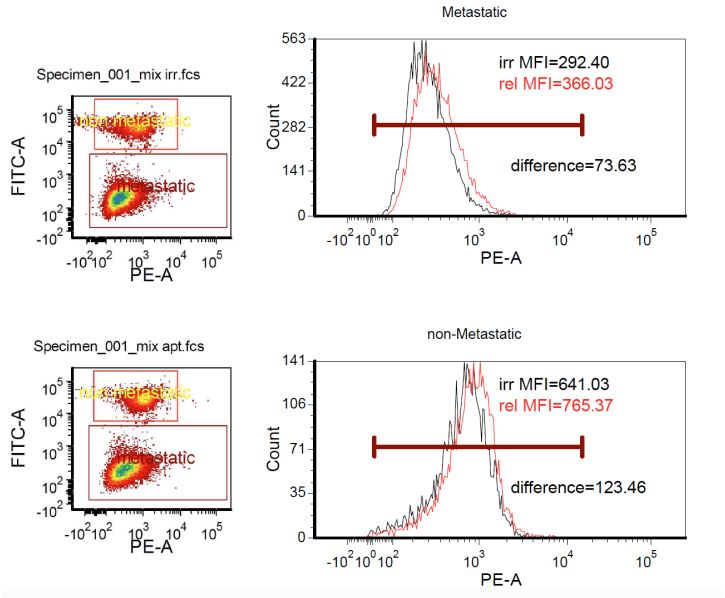

Figure 2. Initial flow cytometry results after 8 cycles. Modest preferential labeling of the metastasis-prone cells at the upper bounds of PE-A signal (upper right). See text.

Task 5: Serial positive and negative selection cycles. The positive and negative selection of RNA aptamers that bind either organ-confined or metastatic LNCaP cells were repeated for 8 cycles. Aliquots of the selected aptamer pools were reserved after each cycle to document amplification. Figure 1 illustrates the successful subtraction of the RNA library, most visibly between cycles 3-5.

Task 5b. FACS validation. Cycle 8 and Cycle 0 pools were fluorescein-labeled and incubated with LNCaP-Pro-5 and LNCaP-LN3, followed by flow cytometry (Figure 2). An irrelevant RNA aptamer pool (irr, shown in black, upper and lower right panels) was used as a negative control. The results suggested that further subtraction cycles be performed using LNCaP-Pro-5 as negative selector for cycles 9-11.
Status: Completed.

Task 6: Next-Generation Sequencing.
Task 6a. With the modestly encouraging results from FACS, we proceeded with next-generation sequencing on the RNA aptamer pools after cycle 11 (replicate samples x 2), using an Illumina HiSeq 2000 sequence detection system. After 11 SELEX cycles, the pools of RNA aptamers from cycles 0, 4, 9, and 11 were sequenced.
Task 6b. High-throughput sequencing data derived from each pool were filtered, clustered, and analyzed to identify core sequence motifs of selected RNA aptamers. Figure 3 shows the qualitative results of sequence analysis on the RNA aptamer pools. Overall Cell-Selex resulted in the reduction of RNA sequence complexity down to 4 major families (Figure 3). Within these, 8 aptamers, representing each of the 4 sequence families, were chosen for further study..
Status: Completed.

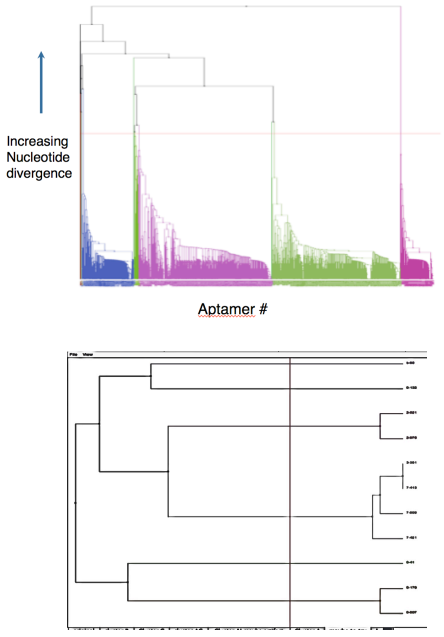

Figure 3. Aptamer NGS and analysis. Sequence analysis of RNA aptamer pools reveals enrichment of 4 families of sequences (upper panel, colored by cluster). Within these families 11 recurring sequences can be identified and their phhyogenetic relationship defined (lower panel).

Task 7. Using selected aptamers for identification of aggressive prostate cancer. Our RNA aptamer pools selectively bound to PC cells prone to aggressive growth and metastasis even in the absence of information about the specific

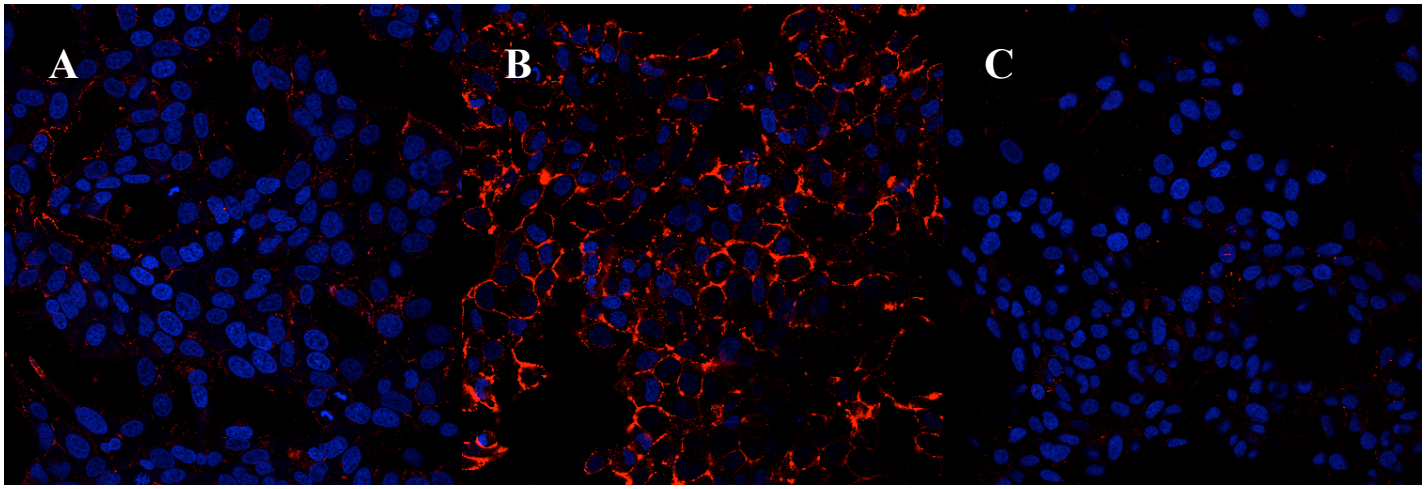

Figure 4. Aptamer 63 specifically labels aggressive prostate cancer cell line. A. LNCaP-LN3 labelled with cycle 0 (pre-selection) aptamer pool. Only background staining is observed. **B.** LNCaP-LN3 labelled with Aptamer 63. Plasma membranes are stained red. C. LNCaP-Pro-5 labelled with Aptamer 63. Background staining is absent. Original magnification: 60x.

protein biomarkers to which they are bound. To begin the process of identifying these proteins and confirming specificity of binding, representative relevant and irrelevant aptamers were labeled with Cy3 and used to stain cells and tumors.

Task 7a: Reactivity of RNA aptamers with PC cells. Cells in tissue culture, LNCaP-ProV (non-metastatic) and LNCaP-LN3 (aggressive) were exposed to Cy3-labeled aptamers as shown in Figure 4. Two aptamers (#41 and #63) showed remarkable specificity for binding to the aggressive LNCaP-LN3 cell surface. The same aptamers were specifically cytotoxic toward LNCaP-LN3 cells after a 3-hour incubation, suggesting an antagonist action on a not-yet identified survival pathway (not shown).

Task 7b: Staining of xenograft tumor tissue.
Xenograft tumors of LNCaP parental, LNCaP-Pro-5 and LNCaP-LN3 were generated in NOD-SCID-gamma immunodeficient mice by direct injection into the prostate gland, and allowed to develop for 6 weeks. Tumors were excised and cryosectioned. Sections were incubated with cycle 0 aptamer pool and Aptamer 63 as above. Figure 5 shows that Aptamer 63 retains its marked selectivity for the aggressive LN3 line in xenograft tumor tissue sections.
Status: Completed.

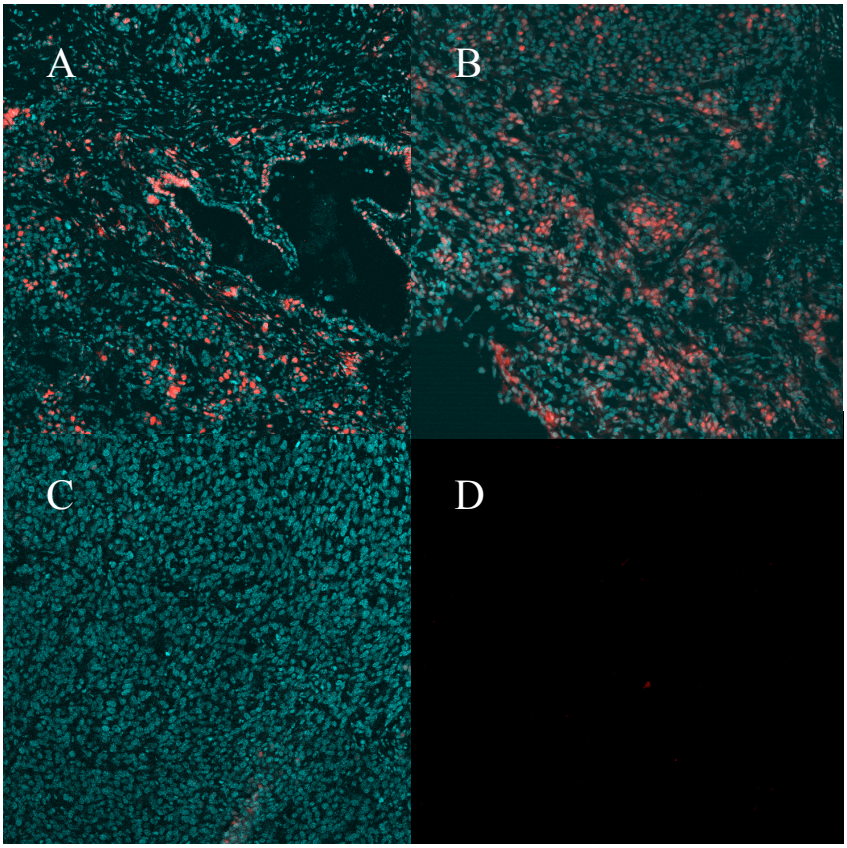

Figure 5. Aptamer 63 specifically stains xenograft tumors derived from the aggressive LNCaP LN3 subclone. A and **B.** 2 sections of LNCaP LN3 xenograft tumors labeled with Aptamer 63. C. LNCaP-Pro-V xenograft tumor labelled with Aptamer 63. Minimal background fluorescence is seen. LNCaP-LN3 xenograft tumor section stained with Cy3-labelled cycle 0 aptamer pool. No fluorescence is seen.

Summary.
Aptamer-based technologies have high potential to identify tumor biomarkers in an unbiased way and to assist in diagnosis and treatment of prostate cancer. We have achieved our major goal of obtaining an aptamer with the abaility to discriminate between two phenotypically distinct subclones of a well-characterized prostate cancer cell line. Our aptamer to specifically binds to and kills the subclone with high metastatic potential.

Future goals and plans include identification of target protein, using pull-down techniques from whole cell lysates, followed by mass spectroscopy, or use of protein microarray in which each protein is transcribed and derived from in vitro immobilized mRNA. In case of a hit in the latter case, the protein target can be identified directly.
In addition, we propose to explore the ability of these aptamers to discriminate between progressive and indolent prostate cancer in tissue microarrays, and ultimately in clinical applications.

What opportunities for training and professional development has the project provided?

The project allowed my junior associate to work with an expert in aptamer technology, Dr. Paolo Serafini, and for both her and me to learn aspects of the bioinformatic analysis required to identify appropriate lead candidates.

How were the results disseminated to communities of interest?

Nothing to report.

This is the final progress report.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?

We have identified a new chemical probe, or aptamer, that specifically recognizes a factor on the surface of aggressive prostate cancer cells. When it binds to the membranes of these cells, they rapidly detach from the culture dish and die. We think that our aptamer will lead us to identify a new marker of aggressive prostate cancers that require treatment, in contrast to slow-growing prostate cancers that can be left alone. This marker is also likely to be a therapeutic target, since binding to it causes cell death. There already exists an aptamer against the general prostate antigen, PSA, that has generated significant interest. Our aptamer is likely to represent a significant additional advance because of its selectivity for aggressive, metastasis-prone tumor cells.

What was the impact on other disciplines?

There is a likelihood that the antigen recognized by our aptamer exists on other cancer cell types. Our aptamer may recognize a general marker of aggressiveness and metastatic potential, as opposed to a marker of prostate origin. This could have diagnostic and therapeutic ramifications for breast cancer and other tumor types.

What was the impact on technology transfer?

(1) We are planning to make an invention disclosure and pursue patent protection for the aptamer sequences.
(2) We have had limited non-disclosing discussions with Ventana Medical Systems, Inc., regarding further testing and commercialization of this aptamer.

What was the impact on society beyond science and technology?

If our results are confirmed, this aptamer could provide doctors and patients a way to decide whether a specific prostate cancer requires treatment or may be followed and watched instead. This would have a significant impact by reducing unnecessary treatment, reducing the attendant morbidity and mortality of treatment, and reducing the cost of treatment.

5. **CHANGES/PROBLEMS:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Nothing to report. All previous changes to the SOW have been reported in May 2014.

Actual or anticipated problems or delays and actions or plans to resolve them

Several parts of this project took longer than anticipated. (1) There were significant delays associated with the transfer of the project to new personnel after the departure of the original PI on this project, Dr. Hnatyszyn. (2) The original plan called for screening of a DNA aptamer library. This library could not be validated, therefore we switched to an RNA aptamer library of known quality. (3) Obtaining a full bioinformatics analysis added more than 3 months to the original time frame due to our collaborators' other commitments. (4) Time required for scaleup synthesis of aptamers and xenograft *in vivo* testing was not factored into our original timeline. This added approximately 3 months.
Plans to resolve: Project is completed.

Changes that had a significant impact on expenditures

Nothing to report. We were able to complete the project without significant additional expenditure by leveraging resources at the institution and through collaborators elsewhere. In particular, costly next-generation sequencing was performed at no charge to us through the University of Modena, Italy. In addition, because the labeled aptamers themselves serve as reagents for imaging, we did not incur the expense of the development or purchase of antibodies against biomarker proteins as described in the original proposal.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal;*

volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to report.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Conference Presentation: S. Speransky, A. De La Fuente, J. Caroli[*], S. Bicciato[*], P. Serafini, N. H. Bishopric (2016). Novel RNA aptamers that specifically bind to metastasis-prone prostate cancer cell surface targets and exert rapid cytotoxicity. Presented at 2016 Zubrod Memorial Lecture and Cancer Research Poster Session, Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine. [*]Center for Genome Research, University of Modena and Reggio Emilia, Modena, Italy

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

We are submitting an Invention Disclosure to UM Technology Transfer this month prior to publication. We plan to submit a full patent if approved by the University.

- **Other Products**

We have generated a novel chemical probe (aptamer) that specifically labels an aggressive, metastasis-prone human prostate cancer cell line. This aptamer will be a useful tool for identifying factors that make prostate cancer more invasive and lethal, and allow it to survive and proliferate. As such it holds promise as both a biomarker and a guide to treatment of prostate cancer, and possibly other cancer cell types.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

1. Name:	Svetlana Speransky
Project Role:	Research Associate
Researcher Identifier	
Nearest person month worked:	12
Contribution to Project:	Ms. Speransky has performed the cell culture and xenograft studies of aptamer specificity, and all of the Cell-Selex panning to identify the lead aptamer candidates.
Funding Support:	Flight Attendant's Medical Research Institute (FAMRI)
2. Name:	Nanette Bishopric
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	NIH Commons nbishopric
Nearest person month worked:	6
Contribution to Project:	I designed the strategy for aptamer panning, participated in the choice of libraries, participated in the bioinformatics analysis, chose the lead aptamers and coordinated the the research team efforts.
Funding Support:	Breast Cancer Research Foundation; Flight Attendant's Medical Research Institute (FAMRI); Susan G. Komen Foundation.
3. Name:	Paolo Serafini
Project Role:	Co-Investigator
Researcher Identifier	
Nearest person month worked:	4
Contribution to Project:	Dr. Serafini provided critical advice and assistance with Cell-Selex panning, and carried out the majority of the bioinformatics analysis for identification of promising aptamer candidates.
Funding Support:	DOD-BCRP; Juvenile Diabetes Research Foundation; National Institutes of Health.
4. Name:	James Hnatyszyn
Project Role:	PI (initial)
Researcher Identifier	
Nearest person month worked:	1
Contribution to Project:	Dr. Hnatyszyn provided the original idea and wrote the original proposal for this project.
Funding Support:	Dr. Hnatysyzn is presently an employee of Ventana Medical Systems, Inc.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Organization Name: Center for Genome Research, Department of Life Sciences, University of Modena and Reggio Emilia

Location of Organization: Modena, Italy

Partner's contribution to the project:
Collaboration: performed bioinformatics
In-kind support: performed next-generation sequencing.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

APPENDIX: Sylvester Poster Session Abstract

Tuesday, May 10, 2016 at the Schoninger Research Quadrangle

University of Miami Miller School of Medicine

S. Speransky[1, 3], A. De La Fuente[2], J. Caroli[4], S. Bicciato[4], P. Serafini[2], N. H. Bishopric[1, 3]
[1]Sylvester Comprehensive Cancer Center, [2]Department of Microbiology & Immunology, [3]Department of Medicine, Miller School of Medicine, University of Miami and [4]Center for Genome Research, Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy

Title: NOVEL RNA APTAMERS THAT SPECIFICALLY BIND TO METASTASIS-PRONE PROSTATE CANCER CELL SURFACE TARGETS AND EXERT RAPID CYTOTOXICITY

Background: Prostate cancer is the most common non-skin cancer diagnosed in American men, with about 1 out of every 7 men receiving this diagnosis during his lifetime. For many men, prostate cancer is indolent, while in others, it is aggressive, metastasizing to other tissues of the body before becoming symptomatic. There is a critical need for diagnostic tests that both enable early detection and predict tumor aggressiveness. To address this need, we are utilizing novel technologies to identify, isolate and characterize high affinity nucleic acid oligomers (aptamers) that distinguish between prostate cancers that are likely to remain organ-confined and those with potential to metastasize.

Methods: We performed subtractive RNA Cell-SELEX using as positive selector a highly metastatic subclone of the prostate cancer cell line LNCapLN3 and as negative selectors both the parent cell line LNCapPro5 and a non-metastasizing subclone, to select for surface ligands specific to the aggressive subclone. The pool of RNA aptamers to be used during the Cell-SELEX were PCR amplified from cDNA library from the general template: TCT CGG ATC CTC AGC GAG TCG TCT G-40(N)-CCG CAT CGT CCT CCC TA, (where N represents 40 random nucleotides) and in vitro transcribed. After 11 SELEX cycles, the pools of RNA aptamers from cycles 0, 4, 9, and 11 were sequenced. High-throughput sequencing data derived from each pool were filtered, clustered, and analyzed. 8 aptamers, representing 4 sequence families, were chosen for further study. Representative relevant and irrelevant aptamers were labeled with Cy3 and used to stain LNCaP-LN3 and LNCaP-Pro5.

Results: Two aptamers (#41 and #63) showed remarkable specificity for binding to the aggressive LNCaP-LN3 cell surface. The same aptamers were specifically cytotoxic toward LNCaP-LN3 cells after a 3-hour incubation, suggesting an antagonist action on a not-yet identified survival pathway. We will present new data on the extent to which these aptamers distinguish between LNCaP-Pro5 (indolent) and LNCaP-LN3 (aggressive) in xenograft tumors in mice in vivo.

Conclusion: Aptamer-based technologies have high potential to identify tumor biomarkers in an unbiased way and to assist in diagnosis and treatment of prostate cancer. We have identified RNA aptamers that specifically bind to metastasis-prone prostate cancer cell surface targets, and exert cell-specific toxicity. We propose that these aptamers may help to discriminate between progressive and indolent prostate cancer in clinical applications.